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Rapid Communication

S100 Protein Positive Dendritic Cells in Primary Biliary Cirrhosis and Other Chronic Inflammatory Liver Diseases

Relevance to Pathogenesis?

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A study to determine the location of dendritic cells, in chronic inflammatory liver disease was performed. S100 protein positivity and dendritic cytoplasmic morphology were used to identify dendritic cells. S100 protein positive dendritic cells (S100 + DC) were found inside the basement membrane between biliary epithelial cells of septal bile ducts of livers affected by early stage PBC, but were not present at later stages. S100 + DC also were seen in areas of piecemeal necrosis in chronic active hepatitis of various etiologies. In contrast, intra-epithelial S100 + DC were not found with any consistency in sclerosing cholangitis, secondary biliary cirrhosis, extrahepatic biliary atresia, or chronic liver allograft rejection, all of which are characterized by inflammatory bile duct damage. The possible relevance of DC in the pathogenesis of PBC is discussed. (Am J Pathol 1989, 134: 741-747)

A dysregulated cellular immune response is thought to be responsible for a number of chronic inflammatory liver diseases.¹⁻⁵ Although there are many studies of these disorders based on phenotypic analysis of organ-infiltrating inflammatory cells, little is actually known about the immunopathologic mechanisms responsible for hepatic damage.⁵ Most investigators have focused on T and B lymphocytes and their subsets, macrophages and other lymphocytes.

More recently, studies have looked at major histocompatibility (MHC) antigen expression by parenchymal cells. To date, however, we are aware of only one study investigating the localization of antigen presenting cells (APC) from the dendritic cell lineage in chronic liver disease.⁶

Dendritic cells (DC) are the most efficient accessory cells known.⁷⁻¹³ They are potent stimulators of the mixed lymphocytes reaction,^{7,9,10} play a critical role in the development of delayed-type hypersensitivity responses,¹⁴ and are required for initial activation of unprimed T lymphocytes.¹¹ Therefore, DC play a critical role in the generation of an immune response, particularly during the afferent phase. The following study was performed in an attempt to begin to understand the role, if any, of DC in chronic inflammatory liver diseases by determining their location in such disorders.

Materials and Methods

Case Selection and Light Microscopy

A number of chronic inflammatory hepatic diseases were selected for study (Table 1). Individual cases were chosen from hepatic resection specimens obtained at the time of transplantation in an attempt to represent a temporal spectrum, as much as possible, of each particular disease. The diagnosis in each case was based on a complete clinicopathologic workup of the patient before transplantation. For example, the criteria for primary biliary cirrhosis (PBC) included positive anti-mitochondrial

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Table 1. *Summary of the Staining Results for the Detection of S100+ DCs*

Disease	No. of cases	Intensity	Location
Normal controls	3	0	Rare intra-epithelial DC in large bile ducts.
Primary biliary cirrhosis			
Stage I-II	5	2+	Intra-epithelial and periductal.
Stage III-IV	7	0-1+	Portal/septal
Sclerosing cholangitis	6	0-1+	Periductal* portal/septal
Secondary biliary cirrhosis	7	1+	Periductal*
Extrahepatic biliary atresia	7	0-1+	Among hilar inflammation
Chronic allograft rejection	8	1+	Portal, periductal portal/septal
Autoimmune	3	0-1+	Areas of piecemeal necrosis
Chronic active hepatitis			
Viral type B	3	0-1+	Portal, areas of piecemeal necrosis
Chronic active hepatitis			
Non A, non B	4	0-1+	Portal, areas of piecemeal necrosis
Chronic active hepatitis			

* Cells usually detected among the intense periductal inflammation around large hilar excretory ducts with disrupted luminal integrity.

antibodies at titers greater than 1:80, combined with a consistent clinicopathologic profile.¹⁵⁻¹⁷ Three normal control livers were obtained from organs harvested for transplantation, but unused because the potential recipient died or was found to have an inoperable malignancy.

The tissue was fixed in neutral buffered formalin, embedded in paraffin, sectioned at 4 μ , and routinely stained

with hematoxylin and eosin (H & E). Two to five blocks that demonstrated active inflammation and ongoing disease activity were selected from each specimen. The sections selected were stained with an antibody directed at S100 protein (Dako, Santa Barbara, CA) for the detection of dendritic cells using the avidin-biotin complex method of Hsu and coworkers.¹⁸

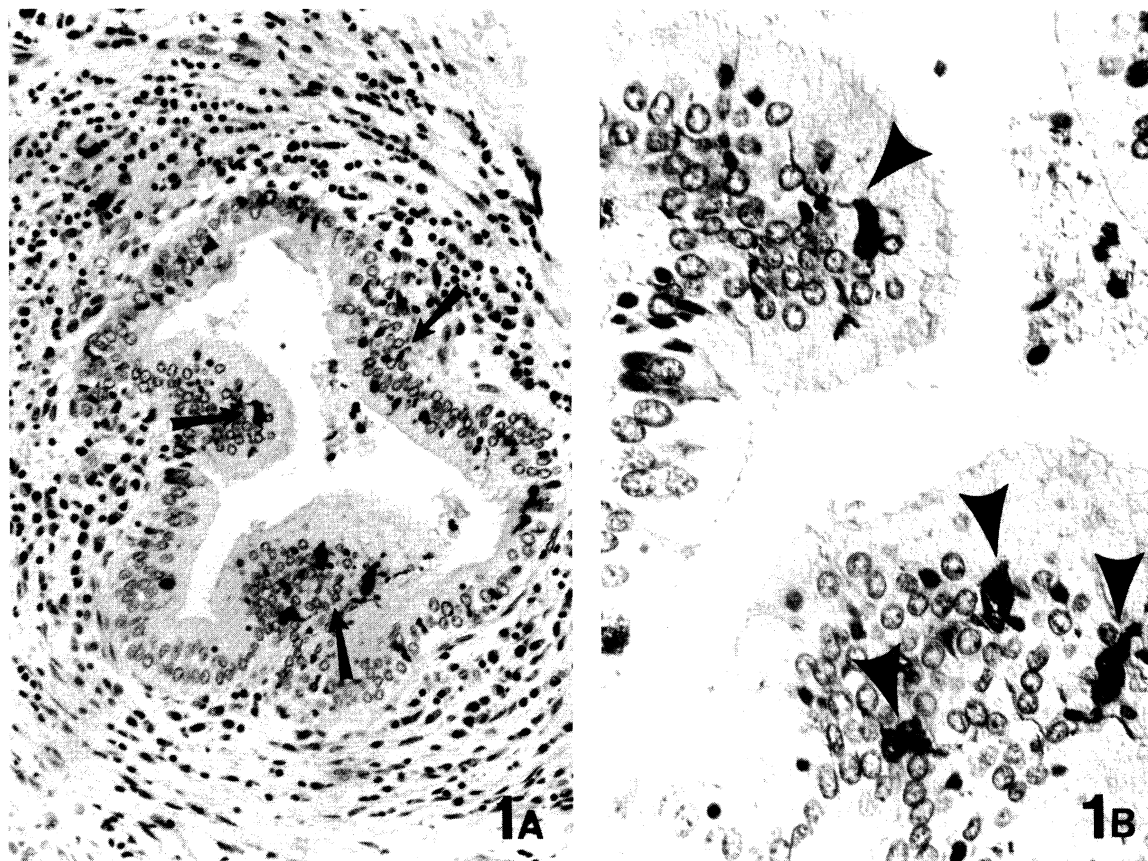


Figure 1A. *Septal bile duct from liver with stage II PBC with early mild inflammatory cell damage. Note the intra-epithelial S100 positive dendritic cells (arrows, $\times 160$; immunoperoxidase for S100 protein). **B:** Higher power of panel A demonstrating the dendritic cytoplasm of S100 positive intra-epithelial cells. (arrow-heads, $\times 400$; immunoperoxidase for S100 protein).*

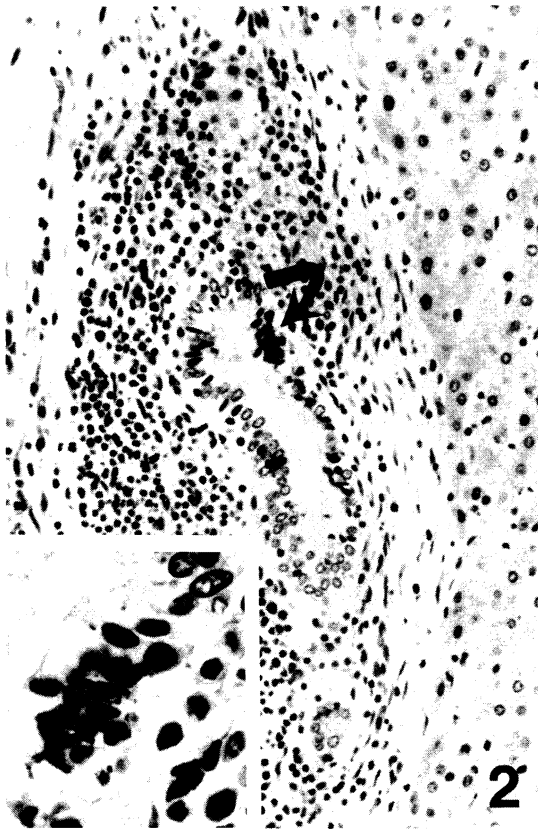


Figure 2. Small septal bile duct from liver with early stage PBC and poorly formed paraductal granuloma (arrow, $\times 160$; immunoperoxidase for S100 protein). Note the intra-epithelial S100 positive DC (arrow) in an area of early duct damage and inflammation (inset, $\times 500$; immunoperoxidase for S100 protein).

For the purpose of this study, a dendritic cell was defined as a S100 protein positive cell with a roughly oval but irregularly shaped nucleus and a cytoplasm that formed flamelike extensions between adjacent cells (dendritic). Cells in which the nucleus could not be identified or lacked dendritic processes, but were S100 protein positive, were enumerated but not considered as true dendritic cells.

The presence, relative number, and location (lobular, portal/septal, periductal, intraepithelial, or in areas of active piecemeal necrosis) of dendritic cells were separately evaluated by four different pathologists and semiquantitatively recorded as follows: none; 1+ (0 to 2/hpf); 2+ (2 to 6/hpf); 3+ (>6 /hpf).

Results

Identification of S100 Positive Cell Populations

Peripheral nerve trunks and adipocytes from hilar sections were uniformly S100 positive in the normal controls and

diseased organs and served as internal controls. Occasionally, glandular epithelial cells in the side pouches of large hilar excretory ducts were also S100 positive. The remaining parenchymal elements of normal and diseased organs were negative.

Three basic types of inflammatory cells were S100 positive but constituted a distinct minority in all the diseased livers. The normal control livers were generally devoid of inflammatory cells except for rare S100 positive DCs in the epithelium of large septal bile ducts. First, rare small round S100 positive cells resembling inactive lymphocytes were seen among the portal inflammation in all disorders. A second distinct population of S100 positive cells resembled macrophages with larger oval, angular, and clefted reniform nuclei, but without definite dendritic cytoplasmic processes. Finally, S100 positive cells with angular clefted nuclei and a dendritic-shaped cytoplasm extended between adjacent cells were the focus of this study.

Intensity and Localization of S100 Positive Cells in Chronically Diseased Livers

S100 positive macrophages and DC were most numerous in the "biliary" diseases (eg, primary biliary cirrhosis,

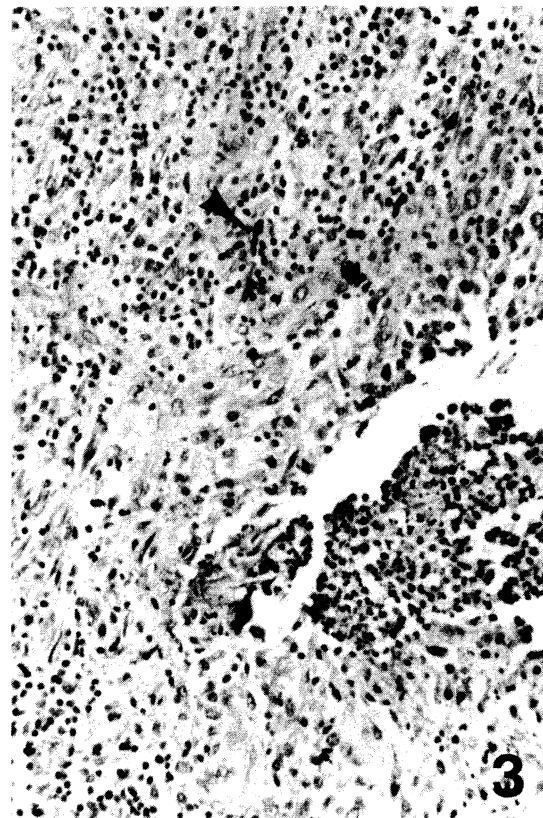


Figure 3. Disrupted septal bile duct from livers with secondary biliary cirrhosis with S100 positive cell (arrow) in the surrounding inflammation (arrows, $\times 160$; immunoperoxidase for S100 protein).

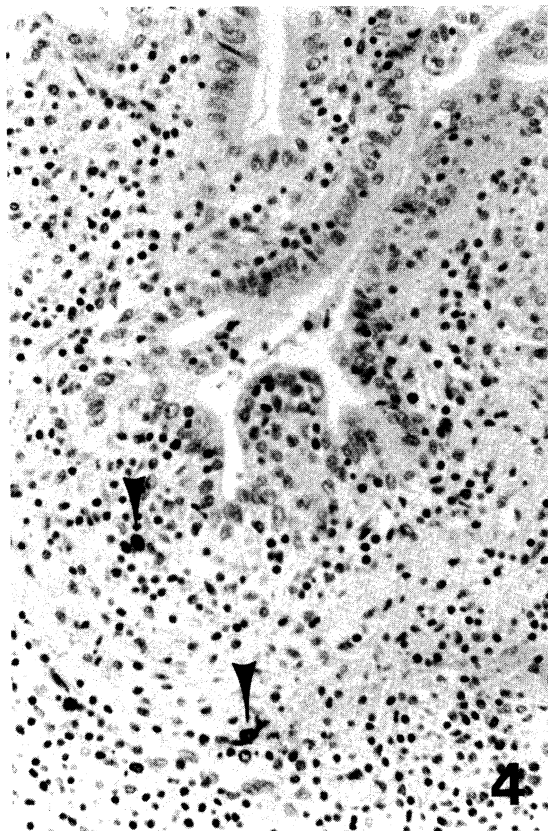


Figure 4. Septal bile duct from liver allograft with chronic rejection. Note the periductal S100 positive cells (arrows) but lack of similar cells in the epithelium. ($\times 160$, immunoperoxidase for S100 protein).

sclerosing cholangitis, secondary biliary cirrhosis, biliary atresia, and chronic allograft rejection) when compared with all other chronic liver diseases studied. Certain generalizations regarding the presence of S100 positive cells could be made. First, as would be expected, S100 positive cells were present only in areas of inflammation. Second, the S100 positive cells were more numerous in the earlier, precirrhotic phases of the disorders when compared with cirrhotic livers. Finally, the S100 positive cells were limited to the portal/septal, intraductal and periductal regions, whereas the lobules were devoid of such cells.

Biliary Disorders

The most striking observation in this group of patients was the intra-epithelial (ie, inside the basement membrane) localization of S100 positive DC in areas of the livers with stage I to II and II to III PBC. These cells were usually localized in septal bile ducts of 100 to 200 μ in diameter showing evidence of early inflammatory cell damage (Figures 1 and 2), which are the initial target structures in early PBC.^{16,17,19} Smaller ducts were less

likely to contain these cells. S100 positive DC and macrophages also were easily identifiable in the periductal region of portal tracts containing florid duct lesions, both within and separate from portal granulomas. It must be reemphasized, however, that although the S100 positive cells were seen in the areas of active inflammation, they constituted a distinct minority population. S100 positive DC also were seen at the limiting plate in the areas of ongoing piecemeal necrosis, but not within the lobule. Livers with later stage PBC (III and IV), however, were generally devoid of DCs.

Livers affected with sclerosing cholangitis also contained occasional intra-epithelial S100 positive DC, but were fewer in number and less prevalent than in livers with PBC. Periductal S100 positive DC and macrophages seen among the intense inflammation around disrupted large hilar ducts were most characteristic of this disorder. Expanded fibrotic portal tracts from the periphery of the liver were generally devoid of S100 positive cells. The findings in secondary biliary cirrhosis were most similar to those seen in sclerosing cholangitis, but usually without intra-epithelial S100 positive DC (Figure 3). More peripheral sections of the livers were devoid of S100 positive cells. Few S100 positive DC were seen among the hilar inflammation in extrahepatic biliary atresia.

Intra-epithelial DCs were not found with any frequency in the allograft livers with chronic rejection; however, periductal S100 positive DC and macrophages could be identified (Figure 4). It should be noted that these organs were largely devoid of small bile ducts, which is characteristic of chronic rejection. Septal ducts, similar to the size damaged in the early stages of PBC, were inflamed but easily identified, and lacked the intraepithelial DCs.

"Nonbiliary" Diseases

Livers from patients with autoimmune, viral type B and non-A, non-B chronic active hepatitis were similar to each other in respect to the location, intensity, and type of S100 positive cells. In general, S100 positive cells were less numerous in these disorders and did not demonstrate any affinity for biliary structures. S100 positive DC and macrophages constituted the majority of identified cells and were found at areas of active piecemeal necrosis and within the portal tracts/septal, whereas the lobules were devoid of such cells.

A summary of the results for the location and intensity of S100 positive DC in the various disorders studied is shown in Table 1.

Discussion

The combination of S100 protein positivity and cytoplasmic dendritic morphology are phenotypic features char-

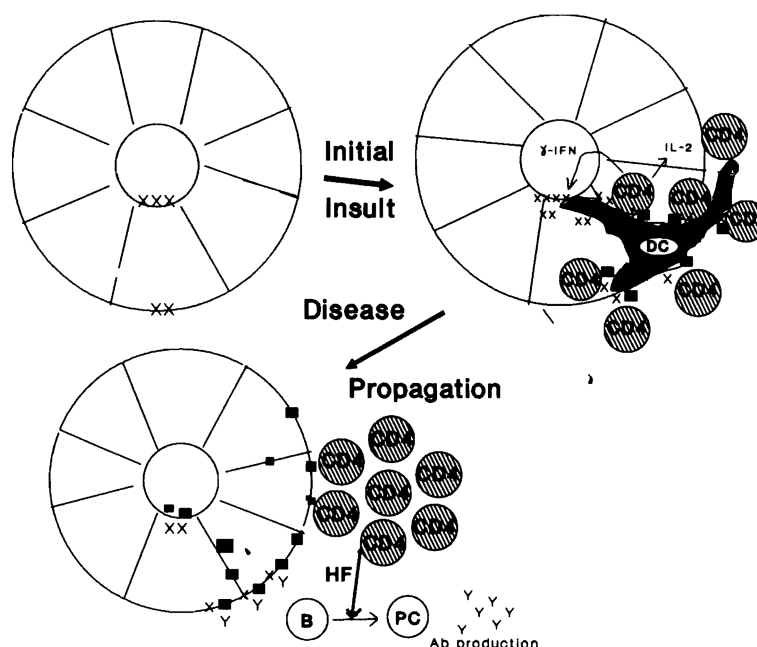


Figure 5. Schematic of hypothesis to explain the possible role of dendritic cells in the pathogenesis of PBC. IFN, gamma interferon; X, target antigens; DC, dendritic cells; B, B lymphocyte; PC, plasma cell; HF, helper factors; ■ HLA-DR.

acteristic of interdigitating reticulum cells (IDC),²⁰ which belong to the dendritic cell family²⁰ and interact closely with T lymphocytes. Such cells or their close relatives are normally present in areas of antigen processing, such as the paracortex of lymph nodes and within the epithelium of the skin and mucosal surfaces of the gastrointestinal tract.²⁰⁻²² The localization of S100 positive DCs in livers with chronic inflammatory diseases but not in normal controls or in the endstages of such disorders suggests that they may play an important role in the initiation and/or propagation of chronic liver disease.⁸

The participation of DC in active piecemeal necrosis in viral type B and non-A, non-B CAH has been reported previously,⁶ and confirmed in this study. Likewise, dendritic cells have been detected in other nonhepatic chronic inflammatory disorders where an autoimmune etiology is suspected.^{23,24} However, identification of S100 positive DC in and around the epithelium of damaged septal bile ducts in early stage PBC is a new observation. Furthermore, their presence in areas of early duct damage, but not in normal livers or in the later stages of PBC, is circumstantial evidence that they may be intimately involved in disease pathogenesis.⁸

It is tempting to speculate that, because S100 positive DCs possess the capabilities needed to trigger an effector inflammatory cascade (eg, ability to activate unprimed T lymphocytes), their role in chronic inflammatory hepatic diseases is crucial. For example, PBC is considered to be a "model autoimmune" disorder,¹⁵ however, the etiology is unknown and pathogenic mechanisms involved in liver damage are poorly understood. Ballardini et al²⁵ documented the aberrant expression of class II MHC antigens

by bile duct cells in livers affected by PBC, as is seen for epithelial cells of other organs affected by autoimmune diseases.²⁶ Parenchymal epithelial cell expression of HLA-DR is thought to enable these cells to function as APCs and present altered self or exogenous antigens to competent T lymphocytes.^{25,26} However, this hypothesis fails to account for the initial stimulus leading to DR expression by parenchymal epithelial cells. Furthermore, it is known that unprimed T lymphocytes require potent APCs (eg, dendritic cells) for initial activation,^{10,11} but once activated, primed T cells are able to interact with other, less potent, antigen presenting cells, such as macrophages, B cells, and perhaps bile duct cells, in generating an immune response.^{11,12} The activated T cells also can produce gamma interferon, which has been shown to induce expression of HLA-DR antigens on bile ducts *in vitro*.²⁷

Perhaps the initiation of the inflammation in PBC is triggered by environmental factors, such as solubilized antigen or immune complexes processed by the hepatocytes or ductal cells and secreted in the bile.^{19,28} The exposure to antigen may then be followed by dendritic cell recruitment or local proliferation, and interaction with CD4+ lymphocytes, which may account for their concentration in the periductal regions of livers with PBC (Figure 5).^{29,30}

Finally, it must be noted that this study suffers from key deficiencies. First, although the organs included herein were chosen because of active disease, a significant proportion were in the mature stages. The exception was cases with early stages of PBC (ie, stage I to II or II to III), where transplantation was performed because of the bone disease associated with this disorder and not the hepatic dysfunction. Second, histopathologic studies are

by nature static, and investigation of disease progression is possible only through multiple samplings from the same organ. Alternatively, studying an animal model would be helpful.³¹ Third, the use of antibodies directed at S100 protein alone, along with dendritic cell morphology, precludes the identification of S100 negative DCs, such as follicular dendritic cells. Such cells may indeed participate in chronic inflammatory liver disease, as suggested by Bardadin and Desmet.⁶ However, the dendritic cell family is a somewhat heterogenous population and all cells do not carry the same antigens, or, if they do, those antigens overlap with ones expressed on the more numerous tissue macrophages. Therefore, we chose a simple system of identification based on cytoplasmic morphology as outlined by S100 protein staining. It should be noted that a previous study by Colucci and coworkers³² failed to detect follicular dendritic cells in PBC, which are another distinct subpopulation of dendritic cells that normally interact with B cells in lymphoid follicles. They used an antibody directed at C3b receptor,³² one that is not present on interdigitating reticulum cells.²⁰ Last, and most important, the documentation of DCs does not prove they are necessary or sufficient for disease occurrence.⁸ Regardless of these shortcomings, we believe that this initial study is suggestive enough to encourage further investigation into the role of DCs in the immunopathology of chronic liver disease.

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